Expression of keratinocyte growth factor receptor correlates with expansive growth and early stage of gastric cancer

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Abstract. The keratinocyte growth factor receptor, also known as KGFR/FGFR2 IIIb, is mainly localized in epithelial cells, and participates in the proliferation of these cells. In contrast, a recent study has revealed that the overexpression of KGFR in salivary adenocarcinoma induces growth inhibition, cell differentiation and apoptosis. We attempted to clarify the expression and role of KGFR in normal and cancerous human gastric tissues and cancer cell lines. Reverse-transcription polymerase chain reaction and Western blot analyses showed KGFR mRNA and its protein expression in NUGC-4, KATO-III and MKN-7 gastric cancer cell lines, but not in the NS-8 cell line. Immunohistochemically, KGFR immunoreactivity was weakly detected in the luminal surface of normal gastric epithelial cells. In addition, KGFR immunoreactivity was strongly detected in the nucleus and cytoplasm of many parietal cells. In gastric cancer tissue, KGFR was expressed in the cell membrane and cytoplasm of cancer cells in 46 of 126 (36.5%) cases. KGFR expression in gastric cancer cells was significantly associated with early-type macroscopic findings, shallow invasion of the gastric wall and expansive growth type. KGFR expression tended to correlate with a good prognosis in gastric cancer. These findings indicate that KGFR expression plays important roles in the differentiation of normal gastric epithelial cells and parietal cell functions. Furthermore, a decreased expression level or the non-expression of KGFR in gastric cancer cells may be associated with the proliferation and invasion of gastric cancer cells and a poor prognosis for the patient.

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Introduction

Gastric cancer is a neoplastic disease with a high incidence and is one of the leading causes of death worldwide, particularly in Asia. The expressions of various growth factors and their receptors in gastric cancer cells have been reported, including epidermal growth factor (EGF), transforming growth factor (TGF)-α, TGF-ß, hepatocyte growth factor (HGF), platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) (1-4).

Fibroblast growth factors (FGFs) 1-23 have been identified in humans and mice. FGF-19 is the human ortholog of mouse Fgf15. Thus, the FGF family contains at least 22 members with molecular masses ranging from 17 to 34 kDa and share a 13-71% amino acid identity (5,6). The biological activities of FGFs are mediated by a family of type I transmembrane tyrosine kinase receptors, FGF receptors (FGFRs) that undergo dimerization and autophosphorylation after ligand binding. There are four FGFRs (FGFRs 1-4); FGFRs 1-3 have two isoforms named IIIb and IIIc (7,8). FGFR-2 IIIb is known as the keratinocyte growth factor receptor (KGFR). The *Kgfr* gene is known to be included in the category of *K-sam*, *Fgfr2* and *bek* genes. KGFR is the product of an alternative splice variant of the *Fgfr2* gene (7). KGFR is specifically expressed in epithelial cells, and FGF-1, FGF-3, FGF-4, FGF-6, the keratinocyte growth factor (KGF/FGF-7) and FGF-10 (KGF-2) have a high affinity to KGFR (8,9).

Mice without the *Fgfr2 IIIb* isoform, but with retaining *Fgfr2 IIIc*, show dysgeneses of the kidneys, salivary glands, adrenal glands, thymus, pancreas, skin, otic vesicles, hair follicles and glandular stomach, and agenesis of the lungs, anterior pituitary glands, thyroid glands, teeth and limbs (10-12). KGFR is expressed in epithelial cells throughout the gastrointestinal tract, and an intraperitoneal injection of KGF enhances normal intestinal epithelial growth (13,14). Regarding cancerous tissue, KGFR expression was previously reported in pancreatic cancer and colorectal cancer cells (15,16). Furthermore, pancreatic cancer cell line growth was stimulated by recombinant KGF administration (17), and dose-dependent cell growth by KGF was observed in well-differentiated colorectal cancer cell lines (18). On the other

hand, a stable transfectant of the *Kgfr* gene of salivary adenocarcinoma cells induced cancer cell differentiation and apoptosis (19). These lines of evidence suggest that KGFR plays important roles in the regulation of epithelial cell and cancer cell functions, such as growth, differentiation and apoptosis.

FGFRs have been implicated in the tumorigenesis of a wide range of cancers, including cancers of the pancreas, breast and stomach and glioblastoma multiforme. K-sam overexpression is associated with the malignant behavior of an undifferentiated type of stomach cancer, such as infiltrative growth and metastasis. Macroscopically, Borrmann type IV or diffusely infiltrative stomach cancer cells are frequently stained for K-sam compared with other types of gastric cancer cell (20,21). Gastric fibroblasts specifically stimulate the growth of scirrhous gastric cancer cells but not that of well-differentiated adenocarcinoma cells. Fibroblasts, particularly gastric fibroblasts, express KGF mRNA, whereas gastric cancer cells do not (22,23). Conversely, scirrhous gastric cancer cells more strongly express KGFR mRNA than well-differentiated gastric adenocarcinoma cells, whereas gastric fibroblasts do not express KGFR mRNA. By immunohistochemical analysis, many undifferentiated types of advanced stomach cancer have been shown to be K-sam-positive, whereas no cases of the K-sam-positive differentiated or intestinal type have been revealed (21). The co-inoculation of scirrhous gastric cancer cells with gastric fibroblasts into nude mice specifically enhances tumorigenicity, compared with that of gastric cancer cells alone (23,24). Furthermore, the histopathological findings of the xenograft produced by co-inoculation with gastric fibroblasts are similar to those of human scirrhous gastric carcinoma (24). All these lines of evidence suggest that KGF, synthesized by stromal fibroblasts and KGFR in cancer cells, has important roles in gastric cancer cell growth, particularly in undifferentiated types of cancer.

To date, the expression of KGFR in normal gastric epithelial cells and the role of KGFR in gastric cancer cells have not yet been completely clarified. In this study, we examined the localization of KGFR in normal gastric epithelial cells and correlation of KGFR expression in gastric cancer cells and the clinicopathological factors.

Materials and methods

Materials. The following were purchased: Histofine Simple Stain Max PO (R) or (M) kits and Simple Stain AP (R) kit from Nichirei (Tokyo, Japan); Immobilon PVDF membrane from Millipore (Yonezawa, Japan); M-PER Mammalian Protein Extraction reagent, a BCA protein assay kit and Super Signal West Pico Chemiluminescent substrates from Pierce (Rockford, IL, USA); horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody from Santa Cruz (Santa Cruz, CA, USA); Isogen from Nippon Gene (Tokyo, Japan); Takara RNA PCR kit (AMV) ver. 3.0 from Takara Biotech (Tokyo, Japan); superfrosted slides with a MAS coat from Matsunami Glass Ind., Ltd. (Osaka, Japan); Vectashield mounting medium containing 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) and fluorescein 5-isothiocyanate (FITC)-conjugated anti-rabbit IgG from Vector Laboratories, Inc. (Burlingame, CA, USA); malinol mounting medium from Mutoh Chemical Co. (Tokyo, Japan). All other chemicals and reagents were purchased from Sigma Chemical Corp. (St. Louis, MO, USA).

Human gastric cancer cell lines. NUGC-4, KATO-III, NS-8, and MKN-7 cell lines were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The cells were grown in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/μl amphotericin B at 37˚C under a humidified 5% CO_2 atmosphere.

Human gastric cancer tissue. Gastric carcinoma tissue samples were obtained from 126 patients (46 female and 80 male) during gastrectomy for gastric cancer at the Center for Digestive Diseases, Second Hospital of Nippon Medical School from 1994 to 1999. The mean age of the cancer patients was 64.4 years (range 26-89 years). According to the criteria of the Japanese Classification of Gastric Carcinoma, there were 54 type 0, 3 type 1, 23 type 2, 28 type 3 and 18 type 4 gastric cancers (25). Histopathologically, there were 29 well-differentiated adenocarcinomas, 29 moderately differentiated adenocarcinomas, 18 poorly differentiated adenocarcinomas (solid type), 19 poorly differentiated adenocarcinomas (non-solid type), 25 signet-ring cell carcinomas, 4 mucinous adenocarcinomas and 2 papillary adenocarcinomas (Table I). Normal gastric tissue samples were also obtained from the surgical margin of the same patient. The tissues were fixed in 10% paraformaldehyde solution (PFA) for 18-20 h and then embedded in paraffin. All experimental procedures were approved by the Human Ethics Committee of the Second Hospital of Nippon Medical School.

Reverse-transcription polymerase chain reaction (RT-PCR). Total RNA extraction was performed using Isogen according to the manufacturer's protocol. Then, cDNA synthesis and PCR were performed using a Takara RNA PCR kit. The primers used for RT-PCR corresponded to nucleotides (nt) 1198-1217 (5'-CAC-TCG-GGG-ATA-AAT-AGT-TC-3') and nt 1330-1347 (5'-CGC-TTG-CTG-TTT-TGG-CAG-3') of the human KGFR cDNA (150 bp, accession no. U11814). The primer pair used for ß-actin was (5'-TAC-ATG-GCT-GGG-GTG-TTG-AA-3') and (5'-AAG-AGA-GGC-ATC-CTC-ACC-CT-3') (218 bp). PCR was carried out using a Takara PCR thermal cycler MP (TP3300, Takara) for 30 cycles, each consisting of 1 min at 95˚C, 1 min at 55˚C and 2 min at 72˚C. The PCR products were analyzed using 2% agarose gel electrophoresis and direct sequencing was performed to confirm the sequences. Total RNA that was not subjected to reverse transcription was employed as the negative control.

Western blot analysis. The anti-KGFR antibody used was an affinity-purified rabbit polyclonal antibody raised against a peptide corresponding to amino acids of the human KGFR protein as previously reported (26). The proteins from the gastric cancer cell lines were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and the separated proteins were transferred to Immobilon PVDF transfer membranes, which were then incubated for 16 h with the anti-KGFR antibody.

Table I. Clinicopathological factors.

Total	126
Gender	
Female	46
Male	80
Age (years)	
<50	18
51-60	21
61-70	45
>71	42
Macroscopic findings	
0	54
1	3
2	23
3	28
4	18
Tumor size (cm)	
<2	28
2-5	50
5-10	36
≥10	12
Histopathological type	
Well-differentiated adenocarcinoma	29
Moderately differentiated adenocarcinoma	29
Poorly differentiated adenocarcinoma (solid)	18
Poorly differentiated adenocarcinoma (non-solid)	19
Signet-ring cell carcinoma	25
Mucinous adenocarcinoma	4
Papillary adenocarcinoma	2
Depth	
m	30
sm	11
mp	12
ss	38
se	30
si	5
Stromal reaction	
Medullary type	20
Intermediate type	47
Scirrhous type	29
Avaluative due to shallow invasion depth[a]	30
Growth pattern	
Expansive type	33
Intermediate type	66
Infiltrative type	27
Lymphatic invasion	
Negative	34
Positive	92
Venous invasion	
Negative	84
Positive	42
Regional lymph node metastasis	
Negative	54
Positive	72
Stage	
IA	39
IB	16
II	14
IIIA	25
IIIB	8
IV	24

[a]According to Japanese Classification of Gastric Carcinoma (25).

The membranes were sequentially washed and incubated with a secondary HRP-conjugated anti-rabbit IgG antibody for 60 min. After washing, the blots were visualized by enhanced chemiluminescence. To confirm the specificity of the positive band, prior to Western blot analysis, anti-KGFR antibody was preincubated with a blocking peptide of KGFR, and then subjected to Western blot analysis using the same protocol as described above.

Immunohistochemistry. The same anti-KGFR antibody used for the Western blot analysis was utilized for immunohistochemistry. Paraffin-embedded sections (3 μm) were subjected to immunostaining using the Histofine Simple Stain Max PO (R) kit for KGFR. Then, endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide in methanol for 30 min. The tissue sections were incubated with the anti-KGFR (1:500 in dilution) antibody in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) for 16 h at 4˚C. The bound antibodies were detected using the Simple Stain Max PO (R) reagent with diaminobenzidine tetrahydrochloride as the substrate. The sections were counter-stained with Mayer's hematoxylin. In some tissue sections, the bound antibodies were detected using the Simple Stain AP (R) reagent with New Fuchsin as the substrate. Counterstaining was not performed in these sections. For Ki-67 immunostaining, the tissue sections were preheated in 10 mM citrate buffer (pH 6.0) for 15 min at 121˚C in an autoclave oven. Then, endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide in methanol for 30 min. The tissue sections were incubated with an anti-Ki-67 (1:100 in dilution) antibody in PBS containing 1% BSA for 16 h at 4˚C. The bound antibodies were detected using the Simple Stain Max PO (M) reagent with diaminobenzidine tetrahydrochloride as the substrate. The sections were slightly counterstained with Mayer's hematoxylin.

Immunofluorescence staining and confocal laser microscopy. The same anti-KGFR antibody used for the Western blot analysis and immunohistochemistry was utilized for immunofluorescence staining. The tissue sections were incubated

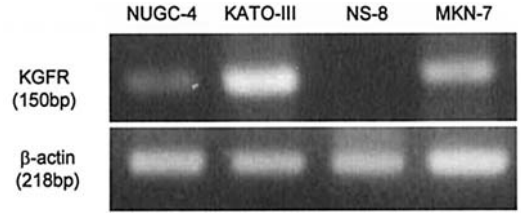

Figure 1. RT-PCR analysis of KGFR mRNA expression in gastric cancer cell lines. Total RNA was extracted from NUGC-4, KATO-III, NS-8 and MKN-7 gastric cell lines, and then cDNA synthesis and PCR were performed. KGFR mRNA at 150 bp was detected in NUGC-4, KATO-III and MKN-7 gastric cell lines (upper panel). ß-actin mRNA at 218 bp was clearly detected in all cell lines (lower panel).

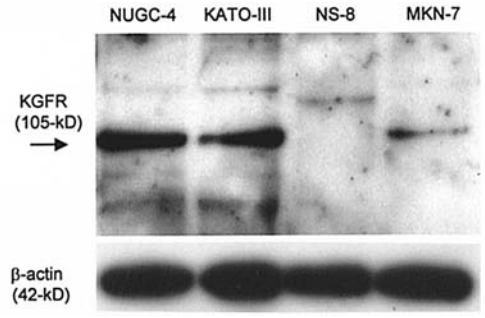

Figure 2. Westen blot analysis of KGFR protein in gastric cancer cell lines. The 105-kDa bands corresponding to KGFR protein were detected in NUGC-4, KATO-III and MKN-7 gastric cancer cell lines (upper panel). ß-actin protein with a molecular weight of 42 kDa was almost equally detected (lower panel).

with the anti-KGFR (1:100 in dilution) antibody in PBS containing 1% BSA for 16 h at 4˚C. Tissue sections were washed with PBS and then incubated with FITC-conjugated anti-rabbit IgG. One hour after incubation, the tissue sections were washed with PBS, and then mounted with Vectashield mounting medium containing DAPI. Fluorescent images were acquired using a confocal laser scanning microscope Digital Eclipse TE 2000-E (Nikon Insteck Co., Ltd. Tokyo, Japan) and a 100x immersion lens (Nikon Palm Apo VC) with blue diode (BD) and argon (Ar) lasers and were analyzed using the confocal microscope Digital Eclipse C1 control software EZ-C1 (version 2.30) (Nikon Insteck). The excitation wavelength for FITC was 488 nm, and emission was selected and recorded using a 500- to 530-nm band-pass filter. In addition, the excitation wavelength for DAPI was 405 nm, and emission was selected and recorded using a 432.5- to 4467.5-nm band-pass filter.

Assessment of KGFR immunostaining in cancer tissue. KGFR immunoreactivity in each tissue section was assessed by two investigators (Tetsuro Matsunobu and Toshiyuki Ishiwata) blind to the clinicopathological features of the tumor or patient survival. Positivity was initially classified according to the percentage of positive tumor cells regardless of staining intensity as follows: positive samples with no tumor cells or positive samples with less than 10% tumor cells were defined as negative, and positive samples with more than 10% cells were defined as positive.

Statistical analysis. Statistical significance was determined using the χ^2 test. Survival rates were calculated for the 126 patients by using the Kaplan-Meier method. The log-rank test was used for univariate analysis to determine differences between curves. Multivariate analysis by Cox proportional hazard regression used only the variables that were significant in the univariate analysis. P-values less than 0.05 were considered statistically significant.

Results

RT-PCR analysis of KGFR in gastric cancer cell lines. To examine the expression patterns of KGFR in human cultured gastric cancer cell lines, RT-PCR was performed. A positive band at 150 bp corresponding to KGFR mRNA was detected in the NUGC-4, KATO-III and MKN-7 cell lines, but not in the NS-8 cell line (Fig. 1, upper panel). ß-actin mRNA was almost equally detected in all cancer cell lines (Fig. 1, lower panel).

Western blot analysis of KGFR in gastric cancer cell lines. To characterize KGFR protein expression in gastric cancer cell lines, total protein was extracted from the NUGC-4, KATO-III, NS-8 and MKN-7 cell lines. Western blot analysis of these cell lines using the anti-KGFR antibody showed a single band corresponding to the KGFR protein at 105 kDa in the NUGC-4, KATO-III and MKN-7 cell lines, but not in the NS-8 cell line (Fig. 2, upper panel). ß-actin protein at 42 kDa was almost equally detected in all four cancer cell lines (Fig. 2, lower panel).

Localization of KGFR and Ki-67 in non-cancerous gastric tissue. To examine the localization of the KGFR protein in human gastric cancer tissue, immunohistochemical staining was performed. In non-cancerous gastric tissue, KGFR immunoreactivity was weakly detected in the cell membrane and cytoplasm in the luminal surface of epithelial cells (Fig. 3A). KGFR was strongly expressed in the gastric pits and lesions of intestinal metaplasia (Fig. 3C and E, arrows). In contrast, Ki-67 immunoreactivity was detected in the nucleus of epithelial cells in the basal and parabasal regions (Fig. 3B, D and F, arrows). In addition, KGFR immunoreactivity was strongly detected in the nucleus and cytoplasm of parietal cells (Fig. 4A and B, arrows). To eliminate the possibility of non-specific staining of the nucleus of parietal cells with DAB, New Fuchsin was used as the substrate for HRP. KGFR was strongly detected in the nucleus and cytoplasm of the parietal cells with New Fuchsin staining (Fig. 4C, arrows). Other techniques, such as immunofluorescence staining and confocal laser microscopy, were performed to confirm the expression of KGFR in the nucleus of gastric parietal cells. Strong KGFR signals were homogeneously detected in the parietal cells (Fig. 4D, arrow). Furthermore, the nucleus that was stained with DAPI was detected in the same section, indicating the KGFR location in the nucleus of parietal cells (Fig. 4E and F, arrow).

Correlation of KGFR expression and clinicopathological factors in gastric cancer. In gastric cancer tissue, KGFR was localized in the cell membrane and cytoplasm of cancer cells and KGFR-positive cells were diffusely distributed in the

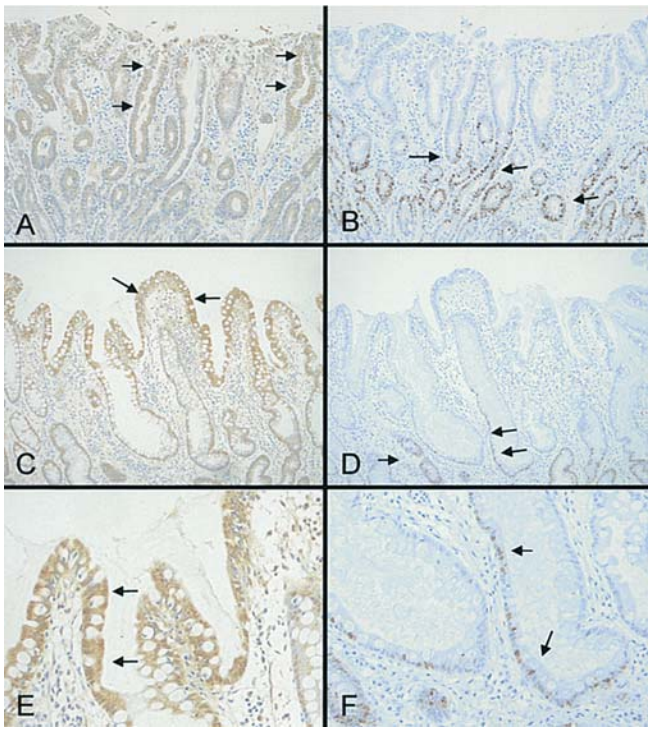

Figure 3. Immunohistochemical analysis of KGFR in non-cancerous gastric tissue. In non-cancerous gastric tissue, KGFR immunoreactivity was weakly detected in the cell membrane and cytoplasm in the luminal surface of gastric epithelial cells (A, arrows). In contrast, Ki-67 immunoreactivity was detected in the nucleus of epithelial cells in the basal and parabasal regions (B, D and F, arrows). KGFR was more strongly detected in the gastric epithelial cells of intestinal metaplastic lesions (C and E). A, C and E, KGFR immunohistochemistry; B, D and F, Ki-67 immunohistochemistry. Original magnification: A-D, x200; E and F, x400.

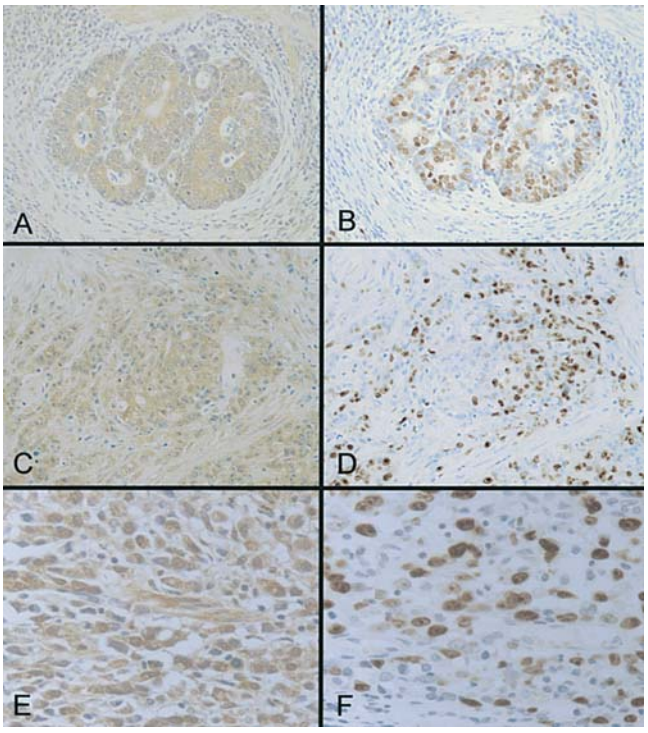

Figure 5. Immunohistochemical analyses of KGFR in gastric cancer tissue. KGFR immunoreactivity was detected in the cytoplasm of gastric cancer cells, classified as well-differentiated adenocarcinoma (A), poorly differentiated adenocarcinoma (C) and signet-ring cell carcinoma (B). Ki-67 was localized in the nucleus of the gastric cancer cells in the serial sections (B, D and F). A, C and E, KGFR immunohistochemistry; B, D and F, Ki-67 immunohistochemistry. Original magnification: A-D, x200; E and F, x400.

cancer cell nests in serial sections (Fig. 5A, C and E, respectively). Ki-67 was localized in the nucleus of the cancer cells (Fig. 5B, D and F).

KGFR was expressed in 46 of 126 (36.5%) gastric cancer cases. The correlation between KGFR expression and clinicopathological factors is summarized in Table II. KGFR expression was significantly associated with early-type macroscopic findings, shallow invasion of the gastric wall,

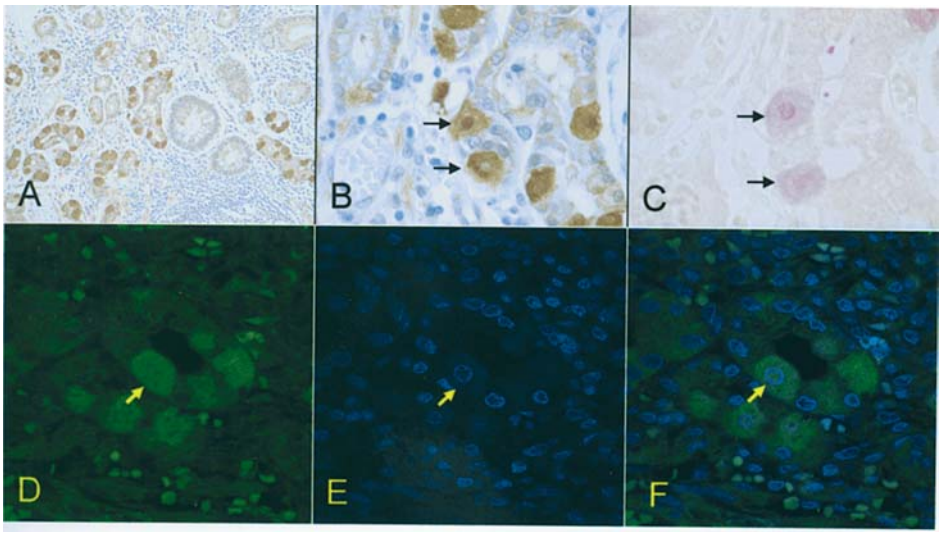

Figure 4. Immunohistochemical analysis of KGFR in parietal cells. KGFR immunoreactivity was weakly detected in the cytoplasm of many parietal cells and strongly detected in some nuclei of the cells (A and B, arrows). This KGFR expression in the nuclei of parietal cells was confirmed using New Fuchsin as the substrate (C, arrows). Strong KGFR signals were homogeneously detected in the parietal cells (D, arrow). The nucleus that was stained with DAPI was detected in the same section (E, arrow). An FITC image and a DAPI image are shown in the same section (F, arrow). Original magnification: A, x200; B and C, x400; D-F, x600.

Table II. Clinicopathological factors and expression of KGFR.

Variables	Expression of KGFR		p-value
	Positive (n=46)	Negative (n=80)	
Gender			NS
Female	16	30	
Male	30	50	
Age (years)			NS
<60	11	28	
>61	35	52	
Macroscopic findings			0.0188
0	26	28	
1, 2, 3, 4	20	52	
Tumor size (cm)			NS
<2	13	15	
≥2	33	65	
Histopathological type			NS
Well-differentiated adenocarcinoma	17	12	
Moderately differentiated adenocarcinoma	7	22	
Poorly differentiated adenocarcinoma (solid)	6	12	
poorly differentiated adenocarcinoma (non-solid)	6	13	
Signet-ring cell carcinoma	8	17	
Mucinous adenocarcinoma	0	4	
Papillary adenocarcinoma	2	0	
Depth			0.0283
m	16	14	
sm, mp, ss, se, si	30	66	
Stromal reaction[a]			NS
Medullary type	9	11	
Intermediate type	14	33	
Scirrhous type	9	20	
Growth pattern			0.0119
Expansive type	19	14	
Intermediate type	18	48	
Infiltrative type	9	18	
Lymphatic invasion			NS
Negative	15	19	
Positive	31	61	
Venous invasion			NS
Negative	35	49	
Positive	11	31	
Regional lymph node metastasis			NS
Negative	24	30	
Positive	22	50	
Stage			0.0149
I	25	30	
II	6	8	
III	13	20	
IV	2	22	

NS, not significant. [a]30 samples (depth m) were excluded, according to Japanese Classification of Gastric Carcinoma (25).

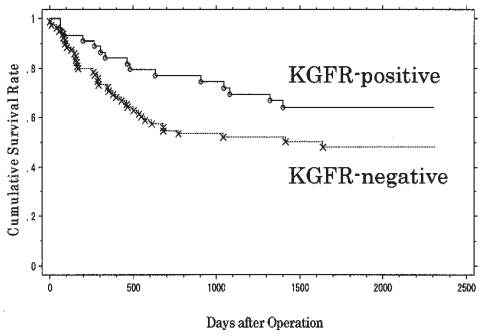

Figure 6. Cumulative survival curves of KGFR-positive and KGFR-negative patients. The overall survival rate of patients who were KGFR-positive was higher than that of patients who were KGFR-negative.

expansive growth type and the early stage of disease (Table II). No appreciable correlation was demonstrated between KGFR expression and either gender, age, tumor size, histopathological type, stromal reaction, lymphatic invasion, venous invasion or regional lymph node metastasis.

Correlation of KGFR expression and patient survival. The overall survival rate of all patients who were KGFR-positive was higher than that of patients who were KGFR-negative (Fig. 6). However, no statistically significant difference was observed between the two groups.

Discussion

In this study, we investigated the KGFR expression in four gastric cancer cell lines and detected it in all four cell lines except the NS-8 cell line. *K-sam* genes have been reported to be expressed in seven of eight gastric cancer cell lines but not in the MKN-1 cell line (20). MKN-1 cells were isolated from a metastatic lesion of an adenosquamous carcinoma of the liver. Although some researchers include *K-sam* genes in the category of *Kgfr, Fgfr2* and *bek* genes, it is considered that *K-sam IIc1* genes actually correspond to *Kgfr* genes (27). To our knowledge, this is the first report on KGFR protein expression in gastric cancer cell lines using a specific antibody against KGFR. NS-8 is a unique adenocarcinoma cell line that produces α-fetoprotein (AFP). The chromosomal localizations of KGFR and AFP (10q26 and 4q11-22, respectively) are different, and the relationship between AFP and the lack of KGFR expression is not clear at present.

In non-cancerous gastric tissue, we detected KGFR-positive sites in the nucleus and cytoplasm of parietal cells in the fundus. The expression of KGFR protein in the nucleus of parietal cells was also confirmed by immunofluorescence staining and confocal laser microscopy. In general, intranuclear receptors are receptors for lipid-soluble ligands and include dioxin receptors and a group of intranuclear steroid receptors (28-31). The intranuclear steroid receptor group in humans includes 48 types of receptor, including those for thyroid hormone, steroid hormones such as estrogen, and lipid-soluble vitamins. These intranuclear steroid receptors are ligand-induced transcription inhibitors and control the expression of specific target genes at the transcription level in response to signals from lipid-soluble ligands. The dioxin receptor is the only known intranuclear receptor for the drug, dioxin, an endocrine disruptor. On the other hand, FGFRs including KGFR are reportedly present in the cell membrane and cytoplasm. The mechanism by which KGFR protein was expressed in the nuclei of gastric parietal cells is not clear. However, intranuclear expression of FGF-2 (basic-FGF) was previously reported, and FGF-1, FGF-2, FGF-3 and FGF-11-FGF-14 were shown to possess nuclear location signals (8,32). Parietal cells were reported to proliferate after the addition of recombinant FGF-7, and the proliferation decreases with the depletion of recombinant FGF-7 (13). These findings suggest that intranuclear KGFR has a specific function in parietal cells with ligands for KGFR.

The Ki-67 antigen is expressed in the nucleus of cells in all active phases of the cell cycle (G1, S, G2 and M phases) (33). In gastric cancer tissue, there was no significant difference between KGFR-positive and Ki-67-positive sites in the cancer cell nests. Previously, we reported that KGFR is localized at the center of cancer cell nests, whereas Ki-67 is localized in the periphery of colorectal cancer tissue (34,35). Furthermore, similar KGFR and Ki-67 localizations were reported in cancer cell nests in pulmonary squamous cell carcinoma. In contrast, KGFR and Ki-67 were reported to be co-localized in lung adenocarcinoma (36). The localization of KGFR in gastric cancer tissue is similar to that of KGFR in lung adenocarcinoma. The higher expression rate of KGFR in well-differentiated adenocarcinoma in the stomach is consistent with those in previous reports, including those observed in colorectal cancer and uterine cervical cancer (35,37).

In this study, we investigated 126 patients and compared the clinicopathological factors between KGFR-positive and KGFR-negative patients. Significant differences were observed between the two groups in terms of macroscopic findings, extent of tumor invasion, growth pattern and clinical stage; for each, more remarkable in less-advanced cancer cases. Likewise, KGFR-positive sites were detected in the cytoplasm of more differentiated types of cancer cell, although no statistically significant difference was found. It was previously reported that more *K-sam* genes are expressed in poorly-differentiated type IV gastric cancer cells and are associated with invasion, proliferation and metastasis (20). The difference between this finding and ours may be caused by the difference between the isoforms of K-sam used, other than KGFR, as mentioned earlier. In addition, KGF is secreted by gastric fibroblasts and is involved in the proliferation of scirrhous gastric cancer. Moreover, the administration of recombinant KGF induced more proliferation in scirrhous gastric cancer (24). These suggest the importance of researching the expression of KGFR ligands, including FGF-1, FGF-3, FGF-4, FGF-6, FGF-7 and FGF-10 in gastric cancer tissue.

In conclusion, KGFR was localized in the luminal surfaces of normal gastric epithelial cells and in the nuclei of parietal cells. The KGFR protein was more frequently detected during expansive growth and in the early stages of gastric cancer. These lines of evidence suggest that KGFR expression correlates with the differentiation of normal gastric epithelial cells and parietal cell functions. The decreased expression level of KGFR in advanced stages of gastric cancer may be associated with the proliferation and invasion of gastric cancer and a poor prognosis for patients.

Acknowledgments

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